

Escape and Aggregation Responses of Three Echinoderms to Conspecific Stimuli

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Abstract. In marine invertebrates, waterborne chemical stimuli mediate responses including prey detection and predator avoidance. Alarm and flight, in response to damaged conspecifics, have been reported in echinoderms, but the nature of the stimuli involved is not known. The responses of *Asterias rubens* Linnaeus, *Psammechinus miliaris* (Gmelin), and *Echinus esculentus* Linnaeus to conspecifics were tested in a choice chamber against a control of clean seawater (no stimulus). All three species showed statistically significant movement toward water conditioned by whole animals or homogenate of test epithelium. *P. miliaris* and *E. esculentus* displayed a statistically significant avoidance reaction, moving away from conspecific coelomic fluid, gut homogenate, and gonad homogenate. *A. rubens* was indifferent to conspecific coelomic fluid, pyloric cecum homogenate, and gonad homogenate but moved away from cardiac gut homogenate. *P. miliaris* was indifferent to gametes, but the other two species were significantly attracted to them. No species showed preference for one particular side of the chamber during trials to balance water flow. These echinoderms can distinguish between homogenates of conspecific tissues that might be exposed when a predator damages the test, and those that may emanate from the exterior surface during normal activities.

Introduction

Predation is a strong selective force, and failing to escape a predator is much more significant in evolutionary terms than are other selective forces such as failure to mate or achieve an optimal energy intake (Lima and Dill, 1990). Animals use a range of cues to detect predators (*e.g.*, visual,

auditory, olfactory, and tactile), but the value of these cues can be limited in aquatic invertebrates. Turbid inshore waters, for example, may render visual cues vague (Mackie, 1975), and currents can concentrate or dilute chemical ones (Weissburg and Zimmer-Faust, 1993). The efficiency of animals tracking chemical cues is greater in calm flowing water and less in rough turbulent flows (Weissburg and Zimmer-Faust, 1994). Movements, scents, or tactile stimuli can warn of predation risk and can originate from the predator itself or from injured or killed conspecifics (Snyder and Snyder, 1970). Such stimuli may trigger escape responses, while others from intact conspecifics may prompt individuals to aggregate in dense groups where the risk of predation to an individual is reduced (Slater, 1985; Zahavi *et al.*, 1999). Such aggregations occur in other animals such as birds, where a high density of individuals within the aggregation or colony has been shown to be related to decreased frequency of attack by predators (Kruuk, 1964).

Adult echinoderms are sedentary organisms and are vulnerable to a range of predators including mammals, birds, fish, invertebrates, and other echinoderms (Mortensen, 1943; Moore, 1966; Mayo and Mackie, 1976; Bernstein *et al.*, 1981). They are, however, able to counter predation by structural and behavioral means such as the use of spines and globiferous pedicellariae, which are minute, forcep-like appendages that can seize and, in some cases, inject venom into the skin of predators (Campbell, 1983). Such mechanisms reduce the ease with which predators can handle their prey, and two categories of behavioral adaptations serve to counter predation in marine invertebrates (Legault and Himmelman, 1993). These are (1) avoidance adaptations that limit the potential number of encounters with predators; and (2) escape adaptations that reduce the risk of predation when a predator has been detected or encountered. Echinoderms provide good examples of both categories.

Received 23 March 1999; accepted 15 May 2001.

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Many echinoderms show avoidance behavior such as burrowing (sand dollars, heart urchins, and some starfish; Lawrence, 1987), covering themselves with a layer of shell and sand (sea urchins; Dayton *et al.*, 1977), or sheltering under rocks and in crevices (Orton, 1914). These habits limit the potential for encounters with surface-moving predators. A remarkable avoidance behavior is shown by the echinoid *Strongylocentrotus droebachiensis*. In summer, it avoids a predator, the diurnal-feeding wolffish *Anarhichas lupus*, by foraging at night (Bernstein *et al.*, 1981); in winter, when the wolffish is less active, it forages throughout 24 hours (Bernstein *et al.*, 1981).

Mauzey *et al.* (1968) described the escape reactions of various invertebrate prey species, including other echinoderms, when they encountered predatory starfishes. One of the clearest escape behaviors is the flight response from predators shown by *S. droebachiensis*, which uses its ventral spines to flee when brought into contact with the starfish *Marthasterias glacialis* (Jensen, 1966). This urchin also flees from water conditioned by crabs or lobsters (Bernstein *et al.*, 1981; Mann *et al.*, 1984). Flight responses in the sea urchin *Diadema antillarum* have been initiated by the body fluids of crushed conspecifics (Snyder and Snyder, 1970; Parker and Shulman, 1986).

Aggregation behavior has been widely reported in echinoderms (Reese, 1966), and in some cases aggregations appear to be related to grazing, detrital feeding, and suspension feeding (Sloan and Campbell, 1982). More recently Levitan *et al.* (1992) showed that aggregation can enhance fertilization success in spawning echinoids. Allee (1927) concluded that echinoderm aggregations are the result of a common response to one or more essential environmental factors, such as food availability, and that they do not represent true social groupings. On the other hand, Bernstein *et al.* (1983) believed that aggregation behavior in *S. droebachiensis* functions as an escape device, reducing the risk to individuals because of the sheer numbers present. These authors considered that predatory crabs would find the entire aggregation of urchins more difficult to handle than single individuals.

Although echinoderms have only simple receptor organs, often made up of a few similar receptor cells without ganglia (Pentreath and Cobb, 1972), many species are sensitive to touch, chemicals, and light, and some may respond to pressure changes and vibrations (Campbell, 1983). Various authors (*e.g.*, Bullock and Horridge, 1965; Chia, 1969; Lepper and Moore, 1998) have described the locomotory and defensive responses of asteroids and echinoids to tactile and chemical stimuli. In most species, the mechanoreceptors and chemoreceptors are located superficially in the test epithelium, from where they can monitor tactile and chemical stimuli (Campbell, 1973; Lepper, 1998). The tube feet are also sensitive to touch and chemicals, and are used to

detect food and prey (Sloan and Campbell, 1982). A range of chemicals, of both low and high molecular weight, initiate responses in asteroids and echinoids (Sloan and Campbell, 1982). Responses to these stimuli range from local reflex reactions in the spines, pedicellariae, and tube feet to fully coordinated responses in which the whole organism moves toward or away from a stimulus source. Experimental analyses by Bullock (1965) and Campbell and Laverack (1968) showed that both the peripheral basi-epithelial nerve plexus of the test and the radial nerve cords of the central nervous systems played a role in mediating these responses.

Many echinoderms possess dermal light receptors, but these are anatomically simple (Yoshida, 1966) and, unlike the eyes of insects, molluscs, and vertebrates, do not form detailed images. Spine movements in response to passing shadows are, however, well known in sea urchins (Millott and Takahashi, 1963).

This paper investigates the effects of waterborne stimuli on three common British species, *Asterias rubens* Linnaeus, *Psammechinus miliaris* (Gmelin), and *Echinus esculentus* Linnaeus. We tested the hypothesis that escape and aggregation responses in sea urchins and starfish are triggered by chemical stimuli emanating from the tissues of conspecific animals and, further, that these responses differ according to the source of the stimulus. The three species tested show broadly similar results. By (1) determining whether these animals display escape and aggregation responses when presented with conspecific stimuli and (2) identifying the body tissue or tissues responsible for producing the effective chemical signal, these experiments add to our knowledge of chemical ecology and the role of chemical stimuli in promoting aggregation or avoidance behavior in mobile animals.

Materials and Methods

Specimens of *Asterias rubens*, *Psammechinus miliaris*, and *Echinus esculentus* were collected from the shore and by dredging from the Isle of Great Cumbrae, Scotland. The animals were transferred to a recirculating seawater system aquarium at Queen Mary, University of London. The species were maintained in separate tanks in a 12-h light:12-h dark regime at 11 °C and 34 ppt salinity. The animals were acclimated for 7 days before testing and were fed mussels *ad libitem* (for *A. rubens*) and other epifauna and epiflora (for the echinoids) brought on small rocks from nearby shores. The size classes of animals that were used in the experiments were as follows: *A. rubens*, R (major radius) = 30–50 mm; *P. miliaris*, 20–35 mm test diameter; and *E. esculentus*, 80–120 mm test diameter.

These animals were tested in a choice chamber (see Fig. 1) based on the design of Mann *et al.* (1984), which was chosen because it allowed the test subject to be simulta-

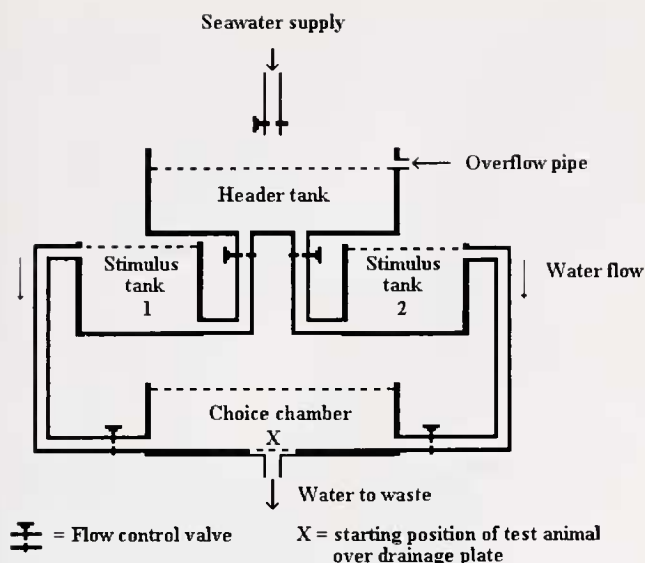


Figure 1. Diagram of the choice apparatus used to test the responses of three echinoderms to waterborne stimuli (not to scale). Internal dimensions of components: header tank 300 mm long, 200 mm wide, and 190 mm deep; stimulus tanks each 175 mm long, 115 mm wide, and 115 mm deep; choice chamber 400 mm long, 160 mm wide, 140 mm deep, and drainage plate diameter 40 mm. Seawater supply to header tank set to overflow constantly; flow tubes to choice chamber set to deliver 0.36 l/min to each side. Flow tubing 8 mm internal diameter.

neously stimulated by two unmixed water bodies. This is impossible in a Y-maze where test animals have to move up the base arm of the Y in a water body containing two elements that may be partly mixed together before the test subjects reach the point of choice. Moreover, some species may move so quickly that they pass into one arm of the Y before making a purely chemically cued choice (Bartel and Davenport, 1956). A significant development in choice apparatus occurred when Pratt (1974) designed a choice chamber to investigate the attraction of prey and the stimulus to attack in the predatory gastropod *Urosalpinx cinerea*. His choice chamber featured two slightly inclined slopes draining centrally in a narrow rectangular chamber and allowed the test animal to be stimulated by two unmixed water bodies at the same time (Pratt, 1974).

In the present work, the header tank, stimulus tanks, and choice chamber itself were all made from clear acrylic plastic. The tubing connecting the tanks (see Fig. 1) was made of flexible plastic with an internal diameter of 8 mm. The header tank acted as the reservoir and supplied the two stimulus tanks with running seawater, which could be adjusted by flow-control valves (see Fig. 1). These two stimulus tanks respectively supplied opposite ends of the choice chamber via 1-m-long flexible plastic tubes also fitted with flow-control valves. Each delivered water to the choice chamber at a rate of about 0.36 l/min. The choice chamber itself was a narrow rectangular trough with a perforated

circular drainage plate, 40 mm in diameter, fitted flush to the center of the tank bottom, for wastewater outflow. The chamber measured 400 mm long by 160 mm wide by 140 mm deep (internal measurements).

The choice chamber was positioned on a wet bench close to the sea urchin holding tank, so quick transfers of experimental animals were possible. Clean uncirculated water was used to feed the apparatus during the experiments. However, beforehand, two dyes—green for the left and red for the right—were added simultaneously to each stimulus tank and the flow valves adjusted so that the left and right water flows met exactly in the center of the drainage plate. “Threads” of dye reached the drainage plate within 2 min of the system being set to run. After 5 min there were no pockets of undyed water in the choice chamber, and a front of dyes was clearly visible, with no mixing, over the chamber drainage plate.

To run the experiments, the system was set with similar flow rates of clean seawater entering each side of the choice chamber, thus reducing any confounding effects of rheotaxis. The test animal was placed in the middle of the drainage plate outlet and allowed to acclimate for 1 min. Then, over the next 10 min, 20 ml of test stimulus extract (see below) was introduced directly into the outflow tube of whichever stimulus tank was in use, at the point the tube left the tank. This inevitably led to dilution, which can be estimated over the 10 min of stimulus application as follows: volume of stimulus, 20 ml; volume of water flowing from stimulus tank to choice chamber, 3600 ml ($= 0.36 \text{ l} \times 10 \text{ min}$) = 1:180. Each stimulus was given as 20 doses of 1 ml each, delivered at 30-s intervals. This delivery rate of stimuli helped ensure that the stimulus had equally permeated all parts of the appropriate half of the choice chamber and that the test animals were exposed to as constant a stimulus as possible. When whole animals were used for the stimulus, a pair of starfish or urchins were placed in the appropriate stimulus tank so that seawater flowed over them on its way to the choice chamber. The response of the test animal during this time was observed and recorded. The following responses were possible:

1. Movement towards the stimulus: the test animal moved fully off the drainage base plate into the water body conditioned by the stimulus. The minimum movement for this to be scored as a response was 50 mm for *A. rubens*, 30 mm for *P. miliaris*, and 60 mm for *E. esculentus*, these distances being such that they brought the animals at least partly off the drainage plate and clearly into one water stream. Test animals achieving less distance than this or not moving off the drainage plate within 30 min were scored as having no response. Nearly all of the responding animals moved the full distance from the center of the drainage plate

to the stimulus inflow point, a distance of 200 mm (Fig. 1).

2. Movement away from the stimulus: the animal moved fully off the drainage base plate into the unconditioned water body.
3. No response: part of the animal remained on the drainage base plate or the animal moved to the sides of the choice chamber such that at least part of its body was in line with the base plate.

After each experiment the choice chamber was thoroughly rinsed and cleaned of all animal debris. The chamber was washed through with clean seawater for 5 min between each test to ensure that all residual stimuli were removed. In addition, the end of the choice chamber into which the stimulus was introduced was alternated for each successive test. This was to eliminate the effects of any inequality of flow between the two sides of the apparatus that might cause the test animals to favor one side over the other. No animal was tested more than once each day. Animals were randomly selected from a pool of 50 individuals kept in separate holding tanks according to species. The same animal could have been selected by chance on successive days; in that case, it was assumed that its response to a stimulus was independent between days. We are not aware of any studies that contradict this assumption for starfish.

Forty different animals were exposed to conspecific stimuli in each of eight experiments. Thus data were analyzed with $n = 40$. For each stimulus tested, animals were drawn from the same pool of individuals. The following hypotheses were tested using different experimental stimuli as shown:

1. That the two sides of the choice chamber gave similar flow rates and volumes so that the test animals did not favor one side over the other. This was tested in experiments when no stimulus was used in either side of the chamber. These experiments acted as controls.
2. That intact, whole conspecifics are attractive or repellent. Here conspecifics were used as the stimulus and were placed in one stimulus tank. There was no stimulus in the other.
3. That test homogenate, composed of spines and epidermal tissues from conspecifics (one starfish as well as one large or three small urchins) was attractive or repellent to conspecifics. This material were scraped into a 50-ml glass beaker and ground up thoroughly in 25 ml of seawater, using a glass rod. The mixture was then stirred to suspend all fine material before being added to one stimulus tank. This was to determine whether the chemical cues active in (2) above resided in the epithelium and skeleton of the test and its appendages. Since movement over hard substrates abrades urchin spines (Campbell, pers. obs.), naked

calcite can be exposed to seawater naturally, and the inclusion of calcite in this homogenate is appropriate.

4. That coelomic fluid was attractive or repellent to conspecifics. A syringe was used to draw off 25 ml of coelomic fluid from an arbitrary number of starfish or sea urchins *via* a small hole in the aboral surface. This was to determine the attractive or repellent effects of coelomic fluid that is released from animals broken open by attacking predators.
5. That gut homogenate was attractive or repellent to conspecifics. Gut tissue was carefully removed from halved asteroid (excluding the pyloric caeca) or echinoid tests (all the gut) and placed in a 50-ml glass beaker. The tissue collected from one starfish or large urchin or from five small urchins was ground up in 25 ml of seawater, using a glass rod. The mixture was stirred well to suspend cells and fragments and was tested to determine whether gut tissue, which is exposed during predator attacks, might release chemical stimuli warning conspecifics of predator behavior.
6. That pyloric cecum homogenate (for *A. rubens* only) was attractive or repellent to conspecifics. This was prepared as for (5) above and for a similar purpose.
7. That gonad extract was attractive or repellent to conspecifics. Gonad tissue was carefully removed from one halved test of *A. rubens* or *E. esculentus* (large echinoid), or from five tests of *P. miliaris* (small echinoid), and ground up in 25 ml of seawater, using a glass rod. The mixture was stirred well to suspend cells and fragments. When ripe, gonad tissue may contribute a major part of the contents of the echinoderm body cavity and may be released and consumed when predators break open echinoderm tests (Ormond *et al.*, 1973).
8. That gametes were attractive or repellent to conspecifics. Both male and female gametes were extracted from one large or three small sea urchins by injecting 0.5 ml of 0.5 M KCl through the peristomial membrane to initiate spawning; the animals shed their gametes within a few seconds of injection. The gametes were collected over the 5–15 min spawning period that followed by inverting the urchin over a 50-ml glass beaker filled with seawater and immersing the gonopores. Male and female gametes of *A. rubens* were extracted using the method of Kantanani (1969), in which 30 g of L-methyladenine was dissolved in 2.5 ml of seawater (0.5 ml per arm). Gametes were shed 60 min after injection, collected in seawater, and stirred immediately before use to keep them suspended. Gametes were tested to see if they would stimulate gregarious behavior, which is thought to be important in increasing fertilization success at spawning (Reese, 1966; Levitan *et al.*, 1992).

The significance of the collected data was examined in two ways, using a log-likelihood test. First the numbers of animals moving toward and away from the stimulus in question for each experiment were pooled and tested against those not responding with movements at all. The null hypothesis predicted a ratio of 50:50. This showed whether a significant number of animals moved in response to the stimuli as opposed to not moving. Second, the number of animals moving toward the stimulus in question for each experiment was compared with the number moving away. Again, the null hypothesis predicted a result of 50:50.

Results

Overall, about 75% of the test animals responded to stimuli in the choice chamber within 5 min of the start of each experiment, and 80% had traversed the full length of one arm of the chamber within 30 min. In the first set of experiments (Figs. 2a–c) log-likelihood tests revealed that, with the exception of *Asterias rubens* (where there was a lack of significant response to coelomic fluid) and *Echinus esculentus* (where there was a lack of response to stimulus-free water), all three species displayed significant behavioral responses to eight experimental treatment stimuli in the choice chamber ($P < 0.001$ – $P < 0.025$).

In the second set of experiments (Table 1 and Figs. 3a–c), starfish and urchins tested when no stimulus was introduced to the apparatus failed to display a significant preference for one side or the other of the choice chamber ($P > 0.05$). This control experiment showed that the apparatus lacked any intrinsic bias that might have encouraged test animals to move more to one side than to the other. It therefore confirmed that subsequent choices made by test animals, in response to introduced stimuli, would be meaningful. It also showed that there was effectively no significant response to the direction of water flow. All three species of echinoderm tested were significantly attracted to whole conspecifics ($P < 0.001$ – $P < 0.025$) and to the homogenates of their tests, spines, and epidermal tissues ($P < 0.001$ – $P < 0.01$) (Table 1 and Figs. 3a–c). The sea urchins significantly avoided water bodies containing coelomic fluid, whereas the starfish showed no significant response to them (Table 1 and Figs. 3a–c). All animals avoided homogenates of conspecific gut tissue (Table 1 and Figs. 3a–c). *A. rubens* did not respond to homogenate of its pyloric caeca or gonads, but both sea urchin species were significantly repelled by conspecific gonad homogenates ($P < 0.001$). *P. miliaris* was not significantly attracted to gametes, whereas both *E. esculentus* and *A. rubens* were (Table 1 and Figs. 3a–c).

Discussion

The results show that *Asterias rubens*, *Psammechinus miliaris*, and *Echinus esculentus* generally respond to water-

borne stimuli derived from conspecifics (Table 1), being mainly attracted by whole animals, test homogenate, and gametes and mainly repelled by coelomic fluid, gut, and gonad homogenates. These findings agree with a number of studies which have shown that echinoderms perceive and react to waterborne chemical stimuli (Dix, 1969; Snyder and Snyder, 1970; Campbell, 1983; Mann *et al.*, 1984; Parker and Shulman, 1986). Because these animals have low visual acuity, tactile and chemical cues must be the chief stimuli received by their sensory systems (Sloan and Campbell, 1982). A distance-mediated chemosensory system was suspected for prey detection in *Asterias forbesi*, but could not be definitively demonstrated (Lepper and Moore, 1995, 1998). However, electron microscopy has revealed concentrations of suitable receptors in external epithelium in this species (Lepper, 1998).

Various workers have demonstrated aggregation of conspecific echinoderms both in the field and in the laboratory (McKay, 1945; Reese, 1966; Broom, 1975; Tegner and Dayton, 1976), and the significant attraction we have described is likely to mediate this. Three hypotheses have been put forward to explain aggregation, namely that echinoderms can benefit from it (1) by optimizing feeding, (2) by better resisting the attacks of predators, and (3) by improving fertilization success at spawning (Bernstein *et al.*, 1981; Moore and Campbell, 1985; Levitan *et al.*, 1992). Aggregations of *A. rubens* were studied by Moore and Campbell (1985), who showed that not only were individual starfish attracted by waterborne scents of conspecifics, but foraging starfish were more attractive than nonfeeding ones. Aggregation in *A. rubens* may therefore be a response to optimize food locations, as was shown by Ormond *et al.* (1973) for *Acanthaster planci* and has been described for other animals (Zahavi *et al.*, 1999). Aggregating behavior of *Strongylocentrotus droebachiensis* was also investigated by Bernstein *et al.* (1981, 1983), who found that this species forms dense feeding and nonfeeding groups of up to 100 individuals per square meter.

P. miliaris, in contrast, is often found in small groups of between 2 and 10 in the field (Campbell, unpubl.). Its gregarious behavior could be an adaptation to group defense of feeding areas, enhancing foraging success by locating other individuals already feeding (Stone *et al.*, 1993). However, the small size of the *P. miliaris* aggregations makes them unlikely to be anti-predator mechanisms of the type described by Bernstein *et al.* (1983) for *S. droebachiensis*, where predation risk to an individual might be reduced by putting other conspecifics between it and potential predators (Hamilton, 1971). Such behavior is known for other animals (Zahavi *et al.*, 1999). Laboratory experiments showed that *S. droebachiensis* aggregated in the presence of unspecified crab and lobster predators that were unable to attack them effectively because they could not encircle the aggregation

Figure 2a

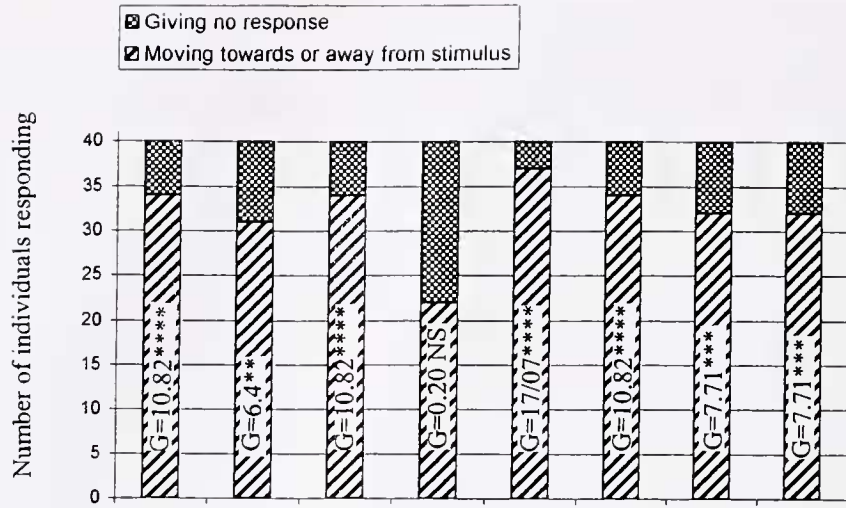


Figure 2b

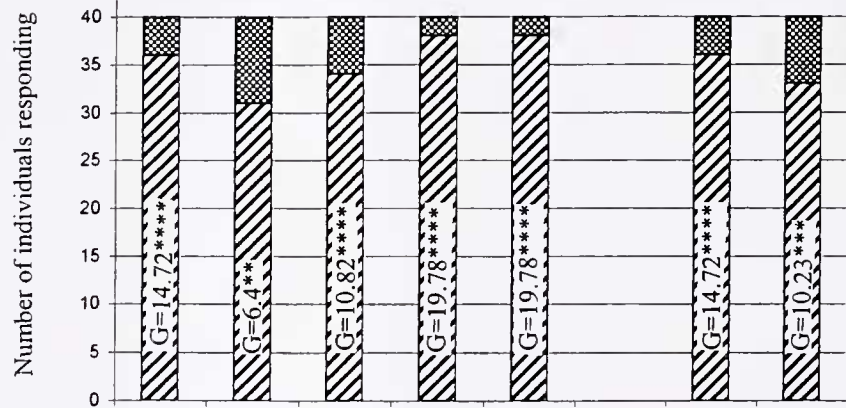


Figure 2c

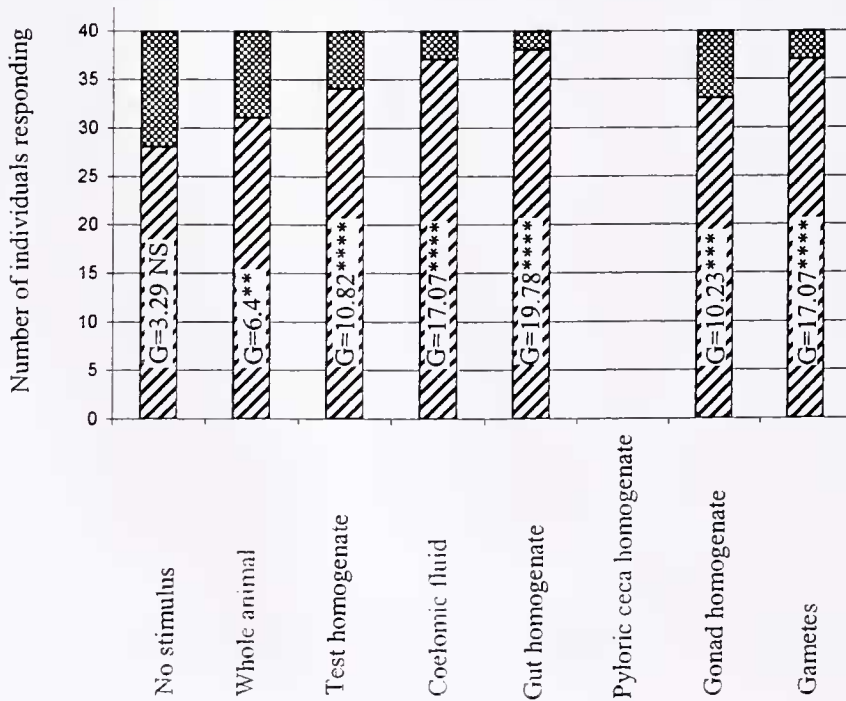


Table 1

Summary of the observed responses of *Asterias rubens*, *Psammechinus miliaris* and *Echinus esculentus* to conspecific stimuli

Stimulus used in choice chamber	No stimulus	Whole animal	Test homogenate	Coelomic Fluid	Gut homogenate	Pyloric cecum homogenate	Gonad homogenate	Gametes
<i>A. rubens</i> moving towards stimulus	18	26	29	10	7	18	16	25
<i>A. rubens</i> moving away from stimulus	16	5	5	12	30	16	16	7
G test value	0.12 NS	15.58****	18.74****	0.18 NS	15.40****	0.12 NS	0 NS	10.76***
<i>P. miliaris</i> moving towards stimulus	17	22	25	5	7	NA	6	19
<i>P. miliaris</i> moving away from stimulus	19	9	9	33	31	NA	30	14
G test value	0.12 NS	5.62**	7.84***	23.08****	16.38****	NA	17.46****	0.76NS
<i>E. esculentus</i> moving towards stimulus	16	24	29	2	3	NA	3	26
<i>E. esculentus</i> moving away from stimulus	12	7	5	35	35	NA	30	11
G test value	0.6 NS	9.86***	18.74****	35.72****	31.68****	NA	25.66****	6.26**

Responses toward stimulus vs. away from stimulus: NS = not significant, $P > 0.05$; * = significant, $P < 0.05$; ** = significant, $P < 0.025$; *** = significant, $P < 0.01$; **** = significant, $P < 0.001$; NA = not applicable.

with their claws (Bernstein *et al.*, 1983). Thus gregarious behavior lowers the intensity of predation and reduces urchin mortality (Bernstein *et al.*, 1983) and, apart from optimizing food locations, these groupings appear to be an effective anti-predator defense.

Orton (1914) found *P. miliaris* living in paired associations of 1 male and 1 female so, alternatively, intraspecific attraction may be explained by spawning aggregation behavior, which is well known for echinoids (Moore, 1966), and which has been shown to increase fertilization success (Levitan *et al.*, 1992). All the specimens in this study were collected in August and maintained at 11 °C. *P. miliaris* is known to breed at Millport from June to August at temperatures of 9–11 °C (Jensen, 1966; Sukarno *et al.*, 1979), so it is likely that the individuals tested in these experiments would be susceptible to factors that might enhance reproductive success. The reasons for aggregation in *E. esculentus* are less clear, as there have been fewer investigations of its social behavior than there have been for *A. rubens* and *P. miliaris*. Aggregations of *E. esculentus* have been noted grazing on algal turf (Forster, 1959), and this species is known to migrate inshore and aggregate for spawning (Elmhirst, 1922; Stott, 1931).

The echinoids in the present study all significantly avoided water conditioned with conspecific coelomic fluid, gut homogenate, and gonad homogenate by moving away

from these stimuli ($P < 0.001$) (Table 1). These escape or alarm reactions are similar to those of *Diadema antillarum*, which fled from fluid extracts of damaged conspecifics using its oral spines as supplementary locomotory organs, in a rapid avoidance reaction (Snyder and Snyder, 1970). Although Snyder and Snyder (1970) were unable to verify their field observations by laboratory experiments, our results are consistent with their findings. *S. droebachiensis* also displays an alarm response to water conditioned by crushed conspecifics and predators (Mann *et al.*, 1984), and this characteristic may explain why natural aggregations of this species decreased in number with increasing abundance of the predatory wolffish *Anarhichas lupus* (Bernstein *et al.*, 1981). Presumably, when this fish attacked an urchin, it released chemicals repellent to other echinoids. Using a choice chamber similar to the one in the present study, Mann *et al.* (1984) showed that 79% of active *S. droebachiensis* moved away from crushed conspecifics, while 70% moved away from predators (*Homarus americanus*). These workers found that when active urchins were exposed to water conditioned by either coelomic fluid, gut, or gonad tissue from conspecifics, 80%–87% of the animals exhibited an alarm response. Mann *et al.* (1984) also showed that when the escape reaction was calculated as a percentage of active urchins it was apparently independent of temperature, whereas the food-seeking reaction was temperature-related.

Figure 2. Number of individuals of each species responding to conspecific stimuli with G-test results and significance code for each stimulus type, (a) *Asterias rubens*; (b) *Psammechinus miliaris*; (c) *Echinus esculentus*. NS = $0 > 0.05$ * = $P < 0.05$ ** = $P < 0.025$ *** = $P < 0.01$ **** = $P < 0.001$.

Figure 3a

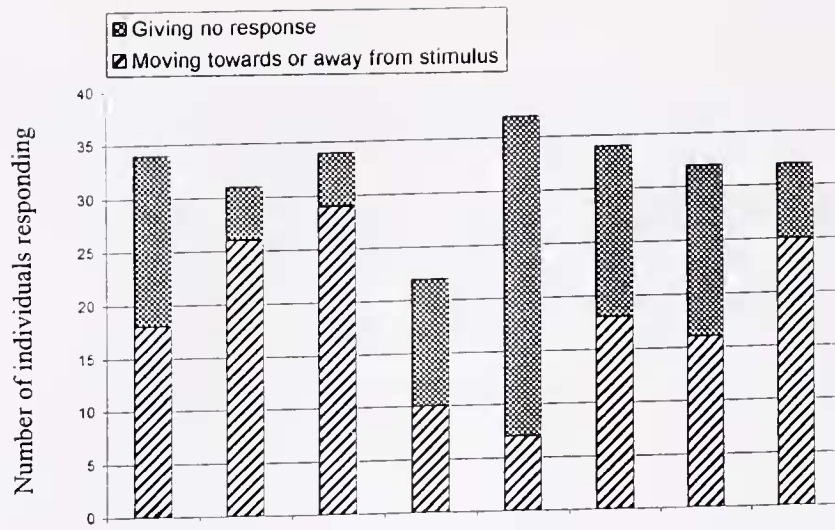


Figure 3b

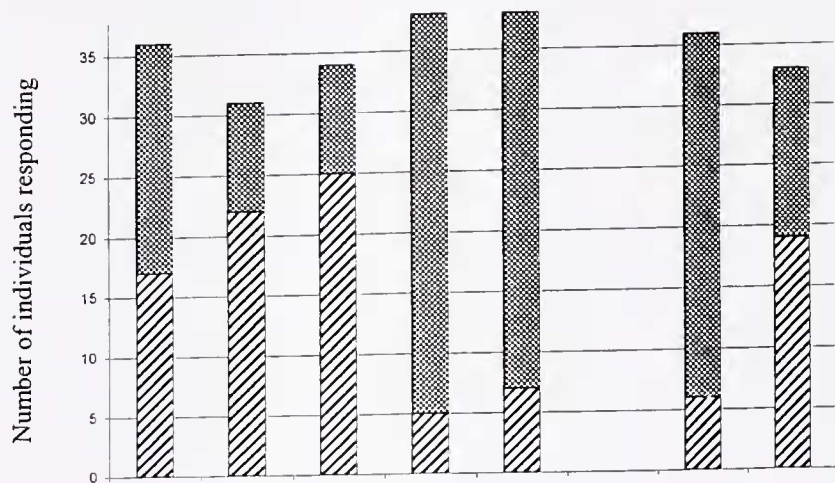
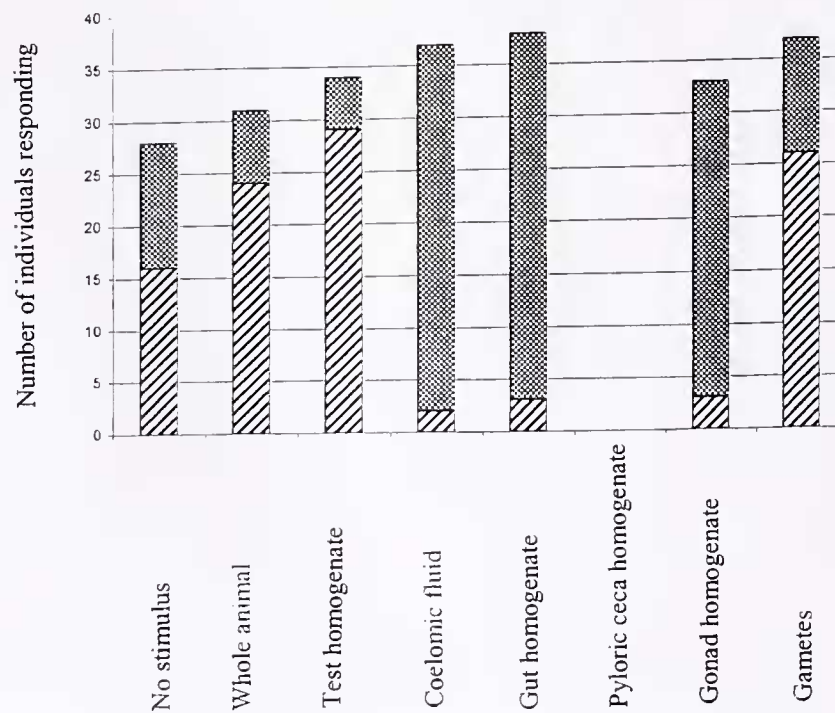


Figure 3c



Thus they suggested that, during the low temperature of winter months, urchin behavior is determined more by the presence of predators than by the distribution of food. The effects of both heterospecific and conspecific body fluids on several species of Caribbean echinoids were examined by Parker and Shulman (1986), who found that the degree of response to conspecific extracts depended on the extent of protection afforded by different microhabitats. Further, there was a correlation between the distance moved by alarmed urchins and the distance they moved from shelter when foraging (Parker and Shulman, 1986). Thus there is a strong implication of predator avoidance in this particular aspect of echinoid behavior.

P. miliaris spends much of its time in protected situations, such as under rocks (Orton, 1929; Jensen, 1966), and makes excursions from these areas to forage on algae and encrusting animals such as sponges, hydroids, bryozoans, and crustaceans (Hancock, 1957). It is during foraging that *P. miliaris* is most vulnerable to predators and that a reliable signal of the presence of a feeding predator will be most advantageous in stimulating the urchin to retreat to safety. The implication of Parker and Shulman's (1986) work may be that the echinoid alarm response to conspecific scents has evolved as an adaptation for escape during foraging periods. The sensitivity to gonad and gut extracts is particularly significant because most predators of echinoids have to break open the test in some way to gain access to the main nutritive elements, the gonads and other viscera. Because *E. esculentus* is a sublittoral species (Orton, 1929), behavioral and ecological observations of this species in the field are less extensive and evaluation of its foraging and escape activities *in situ* is more difficult than for *P. miliaris*, which occurs on the shore. Some echinoderm species form aggregations at spawning time and shed their gametes in synchrony (Moore, 1966). Therefore, why should the urchins be repelled by an extract composed of gonad tissue while not being repelled by gametes, as indicated by the results of this investigation? Possibly the chemical stimulus that caused the urchins to move away from the gonad extract originated in the germinal epithelium or in the nutritive phagocytic tissue. The responses of *A. rubens* to reproductive tissues showed consistencies with those of the echinoids, as starfish were not attracted by gonad homogenate but were by gametes.

In the case of *A. rubens*, gut homogenate (cardiac and pyloric stomach) caused an avoidance response; however, the starfish differed from the urchins in that coelomic fluid did not do so. Possibly *A. rubens*, a known predator and carrion feeder, sometimes acts as a cannibal. Some speci-

mens of *A. rubens* held in the aquaria were seen to eat pyloric ceca previously isolated from other individuals, which accords with Jangoux's (1982) note of cannibalism in this species.

Weissburg and Zimmer-Faust (1993) pointed out that the success of chemically mediated alarm responses in protecting individuals from dangerous situations depends on water turbulence and mixing, because the aquatic environment, as a medium for the transmission of chemical signals, is profoundly affected by hydrodynamics. Every care was taken with our experiments to minimize such disturbances. The use of the dye tests to obtain the correct balance of water flow through the choice chamber allowed us to determine a 10-min regime of stimulus application that subjected the test animals to the most precise stimulus conditions we could obtain. Quantification of the amount of stimulus needed to elicit a response is a desirable but elaborate extension of the experimental procedure, and one that needs to be addressed in future work. Various studies have identified the specific substances to which echinoderms will respond, showing that these animals can react to chemicals such as amino acids, which are present in very low concentrations (Mayo and Mackie, 1976; Sloan and Campbell, 1982; Mann *et al.*, 1984; Lepper and Moore, 1995).

Snyder and Snyder (1970) noted that vinegar produced a flight response in *Diadema antillarum* that was similar to the one initiated by crushed conspecifics. Because crushed heterospecifics had no such effect, they rejected the idea that the escape response was merely due to a change in the chemistry of the water passing over the urchins. Parker and Shulman (1986) found similar results, strengthening the argument for predator avoidance and escape due to extracts from conspecifics. Solandt and Campbell (1998) demonstrated that Caribbean echinoids tested in a choice chamber showed a distinct range of preferences to six algal species, which further supports the idea that these responses are based on choice.

The identification here of clear-cut avoidance and aggregation responses for the two echinoid species shows that they differentiate between, and react to, distinct chemical stimuli under aquarium conditions at a distance of only 1 m. Although the stimulus concentrations are poorly defined, they lie within plausible concentrations for animals living closely together in aggregations or social groups, where one may be seriously damaged by a predator. Our results for *A. rubens* indicate that the distinction between aggregative and repellent effects of various conspecific tissues here is less well defined than it is for the two echinoids, with gut

Figure 3. Number of individuals of each species responding to conspecific stimuli. (a) *Asterias rubens*; (b) *Psammechinus miliaris*; (c) *Echinus esculentus*.

homogenate being the only stimulus that produced a significant avoidance response by the starfish.

Acknowledgments

The authors acknowledge the help and encouragement given by Dr. Maurice Elphick, Dr. Craig Young, and Prof. Paul Tyler. They are indebted to Dr. Carl Smith for his advice on the statistical treatment of the data and to two anonymous referees for their constructive comments.

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